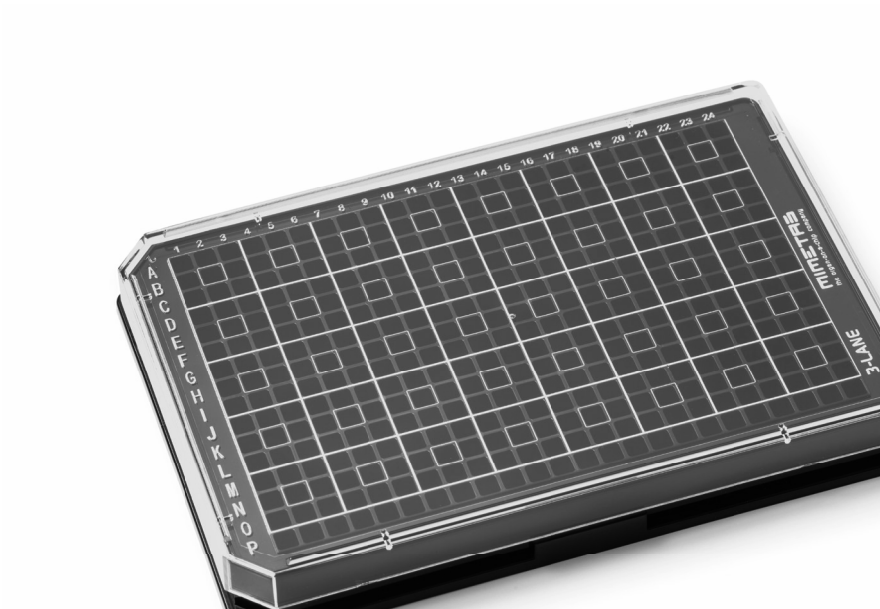
Vasculature and flow in microfluidic systems

Kramer, B.

Chapter 6

Conclusions and perspectives



Conclusions and perspectives

The aim of the research described in this thesis was to develop physiological relevant models that utilize the capabilities of Organ-on-a-Chip technology, such as the incorporation of vasculature and fluid flow. Fluid flow is pivotal in various physiological processes, including the transport of molecules and cells, cell signaling, and tissue development. The hypothesis of this thesis was that Incorporating flow in Organ-on-a-Chip systems is crucial for accurately mimicking the physiological conditions of in vivo organs. The aim was to demonstrate that the introduction of flow into microfluidic devices provides a more realistic simulation of the human body's environment. This is particularly important in the context of drug development, where an accurate prediction of drug responses can significantly streamline the drug development pipeline and therefore lead more efficiently to new therapies, improve the safety of these therapies and reduces costs. This is underscored by recent research highlighting the significance of flow dynamics in microfluidic devices for mimicking in vivo conditions. These studies demonstrate the potential for accelerating the drug development pipeline. [1,2]

Organ-on-a-Chip in drug development

Organ-on-a-Chip is a promising technology to be integrated into the drug development pipeline. The microscale devices are designed to mimic the microenvironment of an organ, including its physical structure, cellular composition and signaling pathways. This thesis discusses the potential of the OrganoPlate as a candidate to enhance the drug development pipeline by serving as an organ modeling tool, with a focus on the incorporation of vasculature and fluid flow into this systems. The emphasis in all the models developed was a balance between high throughput and complex biology, as scalable models are crucial for successful integration of the models into the drug development pipeline. However, the models still need to have adequate physiological relevance to be applicable in drug development.

In **chapter 2**, the aim was to develop a Panreatic Ductal AdenoCarcinoma (PDAC) model in the OrganoPlate 3-lane 40 where chemoresistance with the standard of care (SoC) drug gemcitabine is modeled. Gemcitabine therapy in PDAC patients is not very effective.[3] However, when the PDAC cell line S2-028 in 2D and treated with gemcitabine was cultured, rapid cell death with a low EC50 value was observed. When similar drug exposure in the OrganoPlate model with laminar perfusion flow was performed, increase of the EC50 of three-fold was observed, meaning the cells were more resistant in the three dimensional environment of the OrganoPlate. Furthermore, when the flow pattern was redirected to interstitial flow, another three-fold increase in the EC50 (nine-fold compared to the 2D experiment) was observed. As PDAC is known for its poor drug penetration and high chemoresistance, one would expect an in vitro model to have similar traits. However, in the 2D experiment this expectation was not met, as indicated with the low EC50 value for gemcitabine, suggesting low chemoresistance. In contrast, in the 3D interstitial flow experiment this value was significantly higher, indicating an increase in chemoresistance. This higher resistance better reflects the in vivo scenario, providing a more accurate representation of the challenges associated with therapy development in PDAC. These findings emphasize the importance of recapitulating physiological conditions, such as 3D environments and flow dynamics, into in vitro models to more accurately capture the complexity of drug responses in vivo, not only in PDAC but in other cancers and diseases as well.

Next to drug development, toxicological assessment of compounds is also an important application in Organ-on-a-Chip. In **chapter 3**, the aim was to optimize a microvessel-on-a-chip model for studying monocyte-to-endothelium adhesion under flow. Monocyte adhesion on endothelium occurs when the surrounding tissue is inflamed. Leukocyte adhesion is one of the early stages and hallmarks of atherosclerosis. Primary coronary artery endothelial cells were used to form a microvessel, and an increase of adhesion molecules (ICAM) and oxidative stress (GSH) were observed after application of an inflammatory trigger, while this did not impact the viability of the culture. This inflammatory trigger also significantly

increased monocyte adhesion to the endothelial vessel after 4 and 16 hours. As a proof-of-concept, the application of this model as a toxicology model for cigarette smoke conditioned medium was investigated. An increase in all tested markers with both tested cigarette types was observed, and this was in line with the TNF-α concentrations measured in the samples. Toxicological assessments on the early stage of atherosclerosis development are essential for understanding the impact of various compounds on cardiovascular health. The presented microvessel-on-a-chip model presents a powerful tool for such studies. By revealing increased inflammatory markers and monocyte adhesion in response to cigarette smoke, this model not only confirmed the harmful effects of smoking, but also underscores the utility of organ-a-chip systems in toxicology studies. This assay could not only be applied to study the effects of smoking, but can also be used to study cardiovascular disease in general, and diseases where the inflammation plays a major role, such as rheumatoid arthritis and inflammatory bowel disease.

In **chapter 4** the aim as to optimize a model to assess the effect of human patient serum on the formation and stability of angiogenic sprouts. This was done in the context of systemic sclerosis (SSc), where the endothelium is often dysregulated. Dysregulation of angiogenic sprouts, formed in the Organoplate 3-lane, with cytokines, and subsequently rescue this effect with inhibitors of those cytokines was developed. This was optimized with the use of human serum, as a substitute for the commonly used fetal bovine serum. This approach enabled the investigation of disease scenarios, by using patient derived serum to evaluate the effects on the formation and stability of angiogenic sprouts. In a proof-of-concept study, serum derived from SSc patients had an effect on sprout formation and stability when added during the sprout formation process. This capability to utilize patient-derived serum in Organ-on-a-Chip models underscores a significant advancement in personalized medicine. By capturing the unique cytokine profiles and molecular signatures present in individual patient sera, this model provided a powerful tool for dissecting the pathophysiological mechanisms of diseases like SSc. Furthermore, it offered a promising platform for testing and

developing targeted therapies that are specifically tailored to the molecular and cellular characteristics of each patient, potentially leading to more effective and personalized treatment strategies. Beyond SSc, this approach could be applied to other diseases, such as diabetic retinopathy, where cytokine dysregulation is critical to understanding the disease progression and treatment strategy.

To facilitate targeted drug screening or personalized medicine studies on a high-throughput scale, an automated quantification method of the metric of interest is an necessity. In **chapter 5**, the aim was to develop a novel method to perform a detailed three-dimensional analysis in high-throughput. A vascular angiogenesis model on the OrganoPlate Graft to develop a quantification method to analyze the angiogenic sprout formation in 3D was utilized. Through this method, the progression of vascular beds over time and extracted key metrics, including the count of individual objects and the cumulative sprout volume were successfully measured. This approach could also be applied to a co-culture of endothelial cells and pericytes. The newly developed 3D quantification was compared with a more conventional 2D analysis, and although the quantification methods are comparable in outcome, the 3D analysis method gave more precise results. Having an assay with reduced variability is particularly advantageous for integration into a drug screening setup, as it enables the identification of positive responses to newly tested compounds more quickly. In addition, the 3D dataset comprises a multitude of (spatial and intensity) information on the immunostaining of interest compared to a 2D dataset. Further improvement of the quantification method, such as the inclusion of additional metrics, such as branching points and the z-height distribution of the angiogenic sprouts, may yield crucial information for developing new therapies. This automated quantification approach has potential for applications beyond angiogenesis research, including fields such as tumor microenvironment modeling or neurovascular studies, where spatial organization and precise measurements are critical to understand the complex biological processes and to study therapeutic interventions.

In conclusion, the integration of Organ-on-a-Chip technology into the drug development pipeline offers significant promise to address the high failure rate observed currently in the preclinical stages of the drug development pipeline. A key aspect of this technology is its ability to incorporate fluid flow and vasculature, which are crucial for creating physiologically relevant models. The examples discussed in this thesis illustrate the potential of the OrganoPlate technology to address bridging the gap between traditional in vitro models, animal and clinical studies, offering physiologically relevant insights into drug efficacy and toxicological assessments. Organ-on-a-Chip technology has the potential to transform the drug discovery process by providing more physiologically relevant models. By reducing the reliance on animal testing and improving the translational relevance of preclinical studies, this technology could accelerate the identification of promising drug candidates. Additionally, the thesis showcases the adaptability and growing interest in Organ-on-a-Chip technology among other commercial entities, as evidenced by the successful collaborations in chapters 3 and 4. The emphasis on vasculature and fluid flow in these models underscores their importance in creating more accurate and reliable platforms for drug development.

In this thesis, the hypothesis was that incorporating flow in Organ-on-a-Chip systems is crucial for accurately mimicking the physiological conditions of in vivo organs. The findings in chapters 2, 3, 4 and 5 support this hypothesis by demonstrating that the presence of flow assists in recreating models with complex biology and a relevant microenvironment of specific organs. In chapter 2, the inclusion of laminar and interstitial flow revealed significant differences in drug resistance compared to a 2D culture without flow. In chapter 3, flow through a microvessel was needed to recapitulate the monocyte adhesion process. In chapter 4, the incorporation of vasculature allowed the study of angiogenic sprouts, which highlighted the role of flow in these sprouts maintaining the endothelial health and structure, and allowed to study the effect of patient sera on the stability of these sprouts. Finally, in chapter 5, a vascular angiogenesis

model is presented. Capturing the complexity of the angiogenic sprouts in three dimensions allows for a more precise assay for assessing the effects of compound exposure on the angiogenic sprouts. These findings collectively affirm the hypothesis by showing that flow and vasculature in Organ-on-a-Chip models enhances the physiological relevance. This enhanced physiological relevance is critical for improving the predictability of drug responses and toxicological outcomes, reducing the likelihood of drug candidates failing in clinical trials due to unforeseen issues in more traditional in vitro models and animal studies.

To further enhance the utility of Organ-on-a-Chip models in drug development, several areas could be investigated:

Translatability to In Vivo: While Organ-on-a-Chip models provide a closer representation of in vivo physiology compared to traditional cell cultures, it remains a main challenge to ensure the translatability of findings to human patients. To validate the predictive value of these models, studies need to be conducted that bridge the gap between Organ-on-a-Chip data and clinical outcomes. This could involve the use of validated animal models, with a long-term goal of eventually replacing animal studies, or integrating Organ-on-a-Chip the incorporation of parallel Organ-on-a-Chip studies next to clinical studies to correlate the chip-based predictions with human responses from the clinical trial. Whether Organ-on-a-Chip systems ultimately replace animal models or serve as an addition to the preclinical pipeline will depend on the specific model and the disease or biological process being targeted.

Incorporating Patient-Derived Materials: The ability to assess patient-derived materials, as discussed in chapter 4, offers exciting prospects for personalized medicine and disease modeling. The OrganoPlate's microfluidic design enables the establishment of flow controlled co-culture systems, making it well suited to replicate the complex microenvironments of human tissues. In addition, the low amount of material needed in the OrganoPlate platform, makes it an attractive option for incorporating patient derived materials as the availability of such

materials is severely limited. However, as the patient materials in chapter 4 were incorporated as a proof-of-concept, further research is needed to standardize and validate these approaches, ensuring that the effects observed in Organ-on-a-Chip models accurately reflect patient responses. The scalability and flexibility of the OrganoPlate platform position it as a valuable tool for incorporating patient materials in both preclinical models and the use for personalized therapeutic strategies.

Data Analysis and Integration: As Organ-on-a-Chip models generate complex, multi-dimensional data, robust data analysis methods are essential. As it is rather straightforward to extract data from the Organ-on-a-Chip models on multiple levels, such as analytes from medium, RNA for gene expression analysis and protein expression by immunofluorescent staining, the challenge lies with integrating this data to extract more comprehensive information. In this thesis, computational analyses were somewhat limited, primarily explored in Chapter 5. However, the recent advancements in computing power, algorithms, and artificial intelligence (AI) technologies[4,5] offer promising opportunities to integrate molecular data, such as multi-omics approaches, and correlate these findings with phenotypic observations for a comprehensive insight in the outcomes of the studies.

Automation for High-Throughput Screening: The automation of experimental setups, as briefly discussed in chapter 5, is essential to increase the throughput of drug screening. The footprint of the OrganoPlate and the practical handling make this platform suitable for automation, which was demonstrated recently.[6] High throughput is critical in evaluating a large number of compounds efficiently. Streamlining the process can accelerate drug development and reduce costs, ultimately increasing the chances of identifying successful candidates. To be able to perform high-throughput screening on an assay, such as the assays established in this thesis, some steps need to be taken. First, robust positive and negative controls need to be established for the assay. Second, the assay needs to have proven reproducibility over multiple iterations. Third, quality control must be

implemented, to ensure consistent results over multiple experiments. Fourth, the automated systems must be integrated to manage the addition and removal of media, reagents and compounds across multiple chips simultaneously. Fifth, standardized protocols for imaging and data acquisition need to be developed. Finally, a robust data analysis pipeline is essential to be able to process the large volume of data generated, and allow for the identification of hits and trends in the data. By addressing these steps, the models described in this thesis could be optimized for high throughput applications.

Incorporating these improvements into Organ-on-a-Chip technology holds the potential to revolutionize the drug development pipeline by reducing the attrition rate of drug candidates, minimizing costly late-stage failures, and enabling more precise and personalized therapies. While challenges remain, the continued development of these models in a collaborative effort between academia, industry, and regulatory agencies can help reshape the landscape of drug discovery and ultimately benefit patients worldwide.

References

1. Regmi, S., Poudel, C., Adhikari, R. & Luo, K. Q. Applications of Microfluidics and Organ-on-a-Chip in Cancer Research. Biosensors (Basel) 12, (2022).
2. Wilkinson, J. M. A review of complex in vitro cell culture stressing the importance of fluid flow and illustrated by organ on a chip liver models. Frontiers in toxicology 5, (2023).
3. Koltai, T. et al. Resistance to Gemcitabine in Pancreatic Ductal Adenocarcinoma: A Physiopathologic and Pharmacologic Review. Cancers (Basel) 14, (2022).
4. Wang, D. et al. Human-AI Collaboration in Data Science. Proc ACM Hum Comput Interact 3, 24 (2019).
5. M. Alshater, M. Exploring the Role of Artificial Intelligence in Enhancing Academic Performance: A Case Study of ChatGPT. SSRN Electronic Journal (2022) doi:10.2139/SSRN.4312358.
6. Soragni, C. et al. Phenotypic screening in Organ-on-a-Chip systems: a 1537 kinase inhibitor library screen on a 3D angiogenesis assay. Angiogenesis 1, 1–13 (2023).